

# PATENT SPECIFICATION

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## (54) PROCESS FOR THE PURIFICATION OF A MICRO-ORGANISM PRODUCT

(71) We, THE BRITISH PETRO-  
 LEUM COMPANY LIMITED, of  
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 9BU, a British Company, do hereby declare  
 the invention for which we pray that a patent  
 may be granted to us, and the method by  
 which it is to be performed to be particu-  
 larly described in and by the following  
 statement:—  
 The present invention relates to a process  
 for the purification of a micro-organism  
 product obtained by cultivating a hydro-  
 carbon utilising strain of a micro-organism  
 for example a yeast, on a hydrocarbon sub-  
 strate in the presence of an aqueous nutrient  
 medium and an oxygen containing gas. In  
 particular the invention relates to a process  
 for the removal of lipids and residual hydro-  
 carbons from the micro-organism product.  
 The micro-organism product recovered  
 from hydrocarbon fermentations is con-  
 taminated with residual hydrocarbons.  
 Furthermore it has been found that yeasts  
 cultivated on hydrocarbon substrates, e.g.  
 petroleum fractions have a relatively high  
 lipid content in comparison with yeasts grown  
 on the more conventional carbon, hydrogen  
 and oxygen containing compounds as sub-  
 strates.  
 Processes have been proposed for the  
 purification of this microorganism product  
 which involve a solvent extraction treatment  
 whereby the lipid and/or hydrocarbon con-  
 tent is reduced or eliminated. The object  
 of these purification processes is to provide a  
 micro-organism material which is suitable for  
 use as a foodstuff.  
 Typically in such processes a micro-  
 organism product either dry or in the form of  
 a cream or paste containing water is sub-  
 jected to a wash or more usually a series  
 of washes with an organic solvent e.g. an  
 alcohol or a mixture of alcohol and hydro-  
 carbon, until a material of the desired purity  
 is obtained.  
 When the micro-organism product consists  
 essentially of single cell micro-organisms, e.g.

a yeast, we have found that complex  
 apparatus is required for satisfactory solvent  
 extraction, particularly where, as is usually  
 the case, the micro-organism product is in  
 the form of a powder having a very small  
 particle size. Constraints are set on the type  
 of solvent extraction apparatus which will  
 provide efficient extraction due to the long  
 sedimentation times encountered with pro-  
 duct having a very small particle size.  
 Generally in such a case we have found it  
 necessary to employ multi-stage counter-  
 current extraction incorporating intermediate  
 forced separation stages so as to obtain a  
 material having the required purity.  
 In commercial scale operation it is highly  
 desirable, if not essential for economic  
 reasons alone, to provide a plant in which  
 relatively simple and preferably conventional  
 types of apparatus can be used in the solvent  
 extraction stages for the purification of the  
 micro-organism product.  
 It is an object of the present invention  
 to provide an improved purification process  
 for the removal of substances for example  
 hydrocarbons or lipids from a micro-  
 organism product which is suitable for use  
 in relatively simple and preferably con-  
 ventional type of apparatus, for example a  
 simple cylinder with a once through extrac-  
 tion. We have now discovered that the diffi-  
 culties previously mentioned can be over-  
 come by forming the micro-organism product  
 into granules having a specific particle size  
 prior to extraction with solvents.  
 Accordingly the present invention is a  
 process for the purification of a micro-  
 organism product obtained by cultivating a  
 hydrocarbon utilising strain of a micro-  
 organism on a hydrocarbon substrate in the  
 presence of an aqueous nutrient medium  
 and an oxygen containing gas, the process  
 comprising extracting the micro-organism  
 product in the form of granules having an  
 average particle size in the range 50 to  
 500 microns with an organic solvent for  
 hydrocarbons and/or lipids to give a micro-

organism material having a reduced hydrocarbon and/or lipid content or which is free from hydrocarbons and/or lipids.

5 The process can be carried out on a commercial scale in conventional simple single stage extractors of the "Olier" or "Lodige" type. The "Olier" type extractor is commonly used in the refining of sugar.

10 The granulated micro-organism product can be subjected to a heat treatment before extraction with the solvent. The temperature for the heat treatment can be in the range 100°C to 140°C. The time of heat treatment can be in the range 15 to 45 minutes. It is  
15 convenient to use a temperature of about 120°C for about 30 minutes. In some cases such a treatment was found to improve the extraction process.

20 In practice the granules usually have an average particle size in the range 100 to 500 microns. Most suitably the average particle size is in the range 100 to 200 microns and preferably is about 100 microns.

25 The solvent extraction can be carried out at a temperature in the range 60°C to 80°C and preferably 70° to 75°C. The contact time of solvent and granulated micro-organism product can be in the range 30 to 60 minutes and preferably 40 to 50 minutes.

30 In the extraction operation allowed the solvent can be to percolate through the impure granulated micro-organism product under the influence of gravity. This technique can be operated in simple equipment without  
35 the use of a pump which would otherwise be required to force the solvent through the material to be extracted. A suitable apparatus is then known type of horizontal — basket extractor which consists of a rotatable drum  
40 divided by partitions in a vertical plane into a series of compartments. A solvent feed system is provided at the top of each compartment and a perforated hinged plate forms the base of each compartment. The plate can  
45 be opened to discharge the extracted material.

Alternatively the granulated micro-organism product can be moved counter currently to the direction of the solvent feed. A suitable apparatus is a screw conveyor  
50 extractor wherein the granulated micro-organism product is moved counter currently to the solvent which is gravity fed.

55 Some examples of suitable organic solvents are alcohols, ketones, hydrocarbons e.g. hexane or petroleum ether, or chlorinated hydrocarbons.

Typically the alcohols and ketones which can be used in the process are those which have been described in the literature in  
60 relation to the known processes for the purification of micro-organism containing material. For example British Patent number 1,049,065 discloses the use of ethyl alcohol, isopropanol and acetone. Other examples of  
65 useful alcohols are methyl and butyl alcohol.

Water can be added with the solvent or it can be present, at least in part, in the granulated micro-organism product. It is preferred to carry out the extraction treatment in the presence of between 5 and 15 percent  
70 by weight of water in relation to the weight of solvent. The preferred solvent is isopropanol. In practice the maximum weight of water in relation to the weight of solvent present is about 25 percent. Normally less  
75 than 20 percent of water is used.

Where the granulated micro-organism product is substantially free from water it is preferred to treat it with a mixture containing the desired amount of water and solvent. For example it is often convenient to use an azeotropic mixture of isopropanol and water (i.e. in a proportion by weight of 88 percent isopropanol to 12 percent by weight  
80 of water). On the other hand the granulated micro-organism product itself may contain at least a proportion of water, in which case the appropriate proportion of substantially anhydrous solvent may be used.

85 The micro-organism product can be formed into granules having the desired particle size in the following manner. An aqueous cream containing the micro-organism can be dried using conventional methods such as spray drying to form a powder. The powder thus formed can be mixed with  
90 sufficient water to form a granulated mixture and the mixture sifted to recover granules having the desired particle size.

Alternatively an aqueous cream of paste containing the micro-organism, residual hydrocarbon and spent aqueous nutrient medium can be dried to give a powder containing up to about 40 percent by weight  
100 of water on the dry weight of the micro-organism. Suitable drying techniques include spray drying, drum drying or evaporation using a conventional evaporator, or a fluid bed dryer.

The powder thus formed is then rehydrated if the water content is below 20 percent by weight to give a paste having a water content in the range of about 20 to 40 percent by weight on the dry weight of the micro-organism. The paste thus formed  
105 is further dried to a water content of about 5 percent by weight. Conveniently rehydration can be carried out by mixing the powder with the original cream or paste containing the micro-organism.

120 The partially dried paste containing the micro-organism is then sifted, for example by passage through a sieve, to recover granules having the desired particle size.

Alternatively the powder can be compressed to form the granules.

125 The preferred micro-organisms are single celled, for example hydrocarbon utilising yeasts and in particular members of the genus *Candida*, e.g. *C. lipolytica* and *C. tropicalis*. 130

The purified micro-organism material of the present process which has been freed from the whole or part of the contaminant hydrocarbons and/or lipids in accordance with the process hereinbefore described has an improved taste and is valuable foodstuff.

The lipids removed from the micro-organism product can be used as oil additives, for leather fat liquoring and in addition they form a useful source of fatty acids.

The process of the present invention is further illustrate by but not limited to the following Examples.

#### Example 1

A hydrocarbon utilising strain of the yeast, *Candida tropicalis*, was grown in aerated conditions using a heavy gas oil as the carbon substrate and an aqueous solution of nutrient mineral salts. A cream consisting essentially or about 14.7 percent by weight of *Candida tropicalis*, 0.3 percent by weight of residual gas oil and 85 percent by weight of spent aqueous nutrient medium was recovered from the cultivated broth by decantation followed by centrifugation. This cream was passed to a spray drier and dried to give a yeast product in the form of a powder having a water content of about 5 percent by weight. The yeast at this stage had a lipid content of about 13 percent by weight (estimated on the dry weight of the yeast). The powder was mixed with the original cream in a proportion of 59.4 percent by weight of powder to 40.6 percent by weight of cream to give a paste containing 52.6 percent by weight of dry yeast, 2.0 percent by weight of residual gas oil, 7.9 percent by weight of lipids and 37.5 percent by weight of water. The paste was passed to a flash dryer where water was removed to give a partially dried product having a water content of about 5 percent by weight in relation to the dry weight of the yeast. The partially dried paste was then passed to a sieve where it was sifted to recover a granulated yeast product having an average particle size of about 315 microns.

The granulated yeast product thus formed was fed to a Lédige extractor wherein the yeast granules are moved slowly by an Archimedes screw in the solvent in a ratio of one part by weight of granulated yeast to 10 parts by weight of an azeotropic solvent mixture containing 88 percent by weight of isopropanol and 12 percent by weight of water. The yeast was extracted at a temperature of 70°C with one volume of solvent mixture to one volume of granulated yeast. The solvent flow rate was adjusted to give a drainage time of 10 minutes. The extraction was repeated three times after which a yeast material was obtained which was free from residual gas oil and having a total

lipid content on a dry weight basis of 1.2 percent.

#### Example 2

The solvent extraction procedure described in Example 1 was followed using granulated yeast having the following average particle sizes:— 100 microns, 150 microns, 257 microns and 407 microns. The yeast material obtained after solvent extraction was free from residual gas oil and had the following total lipid contents (percent dry weight) respectively:— 1.03, 1.10, 1.20 and 2.3.

#### Experiment by way of comparison

The solvent extraction procedure described in Example 1 was followed using (a) a spray dried yeast powder having an average particle size of less than 50 microns and (b) a granulated yeast having an average particle size of 565 microns. In the case of the yeast powder (a) it was not possible to obtain an extracted material because the solvent did not pass through the powder. In the latter case the level of residual lipid and hydrocarbon was unacceptably high. The lipid content of the extracted granulated yeast material (b) was 3.8 percent by weight. The material still contained residual hydrocarbon.

#### WHAT WE CLAIM IS:—

1. A process for the purification of a micro-organism product obtained by cultivating a hydrocarbon utilising strain of a micro-organism on a hydrocarbon substrate in the presence of an aqueous nutrient medium and an oxygen containing gas, the process comprising extracting the micro-organism product in the form of granules having an average particle size in the range 50 to 500 microns with an organic solvent for hydrocarbons and/or lipids to give a micro-organism material having a reduced hydrocarbon and/or lipid content or which is free from hydrocarbons and/or lipids.

2. A process as claimed in claim 1 wherein the granules have a particle size in the range 100 to 200 microns.

3. A process as claimed in claim 1 or 2 wherein extraction is carried out by allowing the solvent to precolate through the granulated micro-organism product under the influence of gravity.

4. A process as claimed in any one of the preceding claims wherein extraction is carried out in the presence of between 5 and 15 percent by weight of water in relation to the weight of solvent.

5. A process as claimed in any one of the preceding claims wherein the solvent is isopropanol.

6. A process as claimed in and one of the preceding claims wherein the extraction is

carried out at a temperature in the range 60°C to 80°C.

- 5 7. A process as claimed in any one of the preceding claims wherein the granulated micro-organism product is subjected to a heat treatment at a temperature in the range 100°C to 140°C before extraction with the solvent.

- 10 8. A process as claimed in claim 7 wherein the time of heat treatment is in the range 15 to 45 minutes.

9. A process as claimed in any one of the preceding claims wherein the micro-organism is a yeast.

10. A process as claimed in claim 1 and as hereinbefore described with reference to Example 1. 15

11. A process as claimed in claim 1 and as hereinbefore described with reference to Example 2. 20

12. A purified micro-organism material when obtained by the process as claimed in any one of the preceding claims.

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